

THE EFFECTS OF EYESTALK, LEG, AND UROPOD REMOVAL ON THE MOLTING AND GROWTH OF YOUNG CRAYFISH, *PROCAMBARUS CLARKII*

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The effects of eyestalk removal on molt have been studied in decapod crustaceans (Brown and Cunningham, 1939; Abramowitz and Abramowitz, 1940; Smith, 1940), but few crustaceans have been studied during several consecutive molts, probably because of a high mortality. According to Abramowitz and Abramowitz (1940), however, the removal of the eyestalk itself is unlikely to be the reason for the mortality, because some of their blinded crabs survived for 11 weeks after the removal of eyestalks.

In our preliminary experiments (unpublished), 14 of 90 eyestalkless young crayfish survived after more than 10 molts, though the rest died within 4 months. In most cases, the eyestalkless crayfish died at the time of molt or within 1 or 2 days after the molt, probably because of bacterial infection at the time of molt. If this is so, a high mortality might be avoidable to some extent by keeping the container water clean, without disturbing the molting animals.

The molt cycle in Crustacea is thought to be regulated by two hormones, a molt hormone and a molt-inhibiting hormone. It is believed that the X-organ in the eyestalk produces the molt-inhibiting hormone, which is then stored in the sinus gland. Eyestalk removal, therefore, shortens the following intermolt cycle (Brown and Cunningham, 1939; Smith, 1940). The molt-inhibiting hormone inhibits the activity of the Y-organ, which secretes the molt hormone (Passano, 1960). When the eyestalk is removed the Y-organ is no longer inhibited. If the larvae of the prawn *Palaemonetes kadiakensis* are destalked at metamorphosis, they grow larger in size than the untreated ones, without causing any alteration on the duration of the larval instars (Hubschman, 1963).

It is known that leg removal causes precocious molt in land crabs, *Gecarcinus lateralis* (Skinner and Graham, 1970), freshwater shrimp, *Palaemonetes kadiakensis* (Stoffel and Hubschman, 1974), and crayfish, *Procambarus clarkii* (Bittner and Kopanda, 1973). Skinner and Graham (1972) hypothesized that severing a critical number of leg nerves stimulates the precocious molt.

In the present study, the molt interval and the growth rate of the crayfish were studied during five consecutive molts when the eyestalks, the legs or the uropods were removed.

MATERIALS AND METHODS

Specimens of the crayfish, *Procambarus clarkii*, used in this work were collected from ponds in the suburbs of Yamagata. Only crayfish of 8 to 12 mm in length (the length was measured from the tip of rostrum to the hind margin of cephalothorax carapace) that molted once in the laboratory (initial molt) were

chosen as experimental material. They were kept separately, first in polypropylene containers (Lustro ware of Boden Co., $70 \times 85 \times 45$ mm), then in large containers ($105 \times 120 \times 53$ mm) containing dechlorinated tap water at 22 to 23° C when they grew to more than 15 mm in carapace length. They were kept under a photoperiodic light condition of 14-hr light and 10-hr dark. The animals were fed fish food pellets and fallen dead leaves of persimmon. The dead leaves were effective in keeping the animals healthy. When water in the containers was renewed each day, the crayfish were immersed in 1.3% NaCl solution for 20 to 30 sec to prevent infection by bacteria or protozoa.

The crayfish were classified into four experimental groups: (1) untreated intact crayfish as control; (2) crayfish from which a pair of eyestalks was removed; (3) crayfish from which three pairs of the second, third and fourth walking legs were removed, and (4) crayfish from which a pair of uropods was removed. Each group consisted of 23 individuals that had just finished the initial molt in the laboratory, and every operation was performed on the day following this initial molt. The organs were cut off at their bases with scissors. After the third molt following the operation, the regenerated legs or uropods were removed again. Carapace length was measured two days after each molt.

RESULTS

Molting

Molting rate was recorded daily for 230 days after the initial molt. The results are illustrated in Figure 1A-D. The crayfish which died during the course of the experiments were excluded from the data. Four crayfish from the control group, one from the legless group, and one from the uropodless group failed to complete the fifth molt even after 230 days. Since these animals had a reduced fecal output, their failure to molt may have been the result of factors other than hormonal or nervous controls.

Untreated crayfish (controls). Fourteen individuals completed the fifth molt, but three molted four times and one completed only the third molt. The average time required for 50% of the individuals to reach each consecutive molt (T_{50}) was 17 days for the first molt, 50 days for the second, 87 days for the third, 128 days for the fourth, and 164 days for the fifth, after the initial laboratory molt.

TABLE I

The days of the intermolt cycles (molt to molt) in crayfish, Procambarus clarkii.

Molt	Control (14)	Eyestalkless (11)	Legless (20)	Uropodless (20)
Initial to 1st	19.6 \pm 2.7	6.5 \pm 0.2	14.1 \pm 1.0	19.7 \pm 2.3
1st to 2nd	23.8 \pm 2.9	7.1 \pm 0.3	12.6 \pm 0.9	26.9 \pm 2.9
2nd to 3rd	33.6 \pm 4.2	7.5 \pm 0.2	31.5 \pm 3.8	33.6 \pm 4.2
3rd to 4th	31.3 \pm 3.1	8.8 \pm 0.2	21.1 \pm 1.6	29.9 \pm 2.6
4th to 5th	43.2 \pm 6.0	9.2 \pm 0.4	35.2 \pm 4.6	39.0 \pm 3.4

The number of individuals is in parentheses. Data were obtained from crayfish which had completed five consecutive molts. The standard error of the mean is shown.

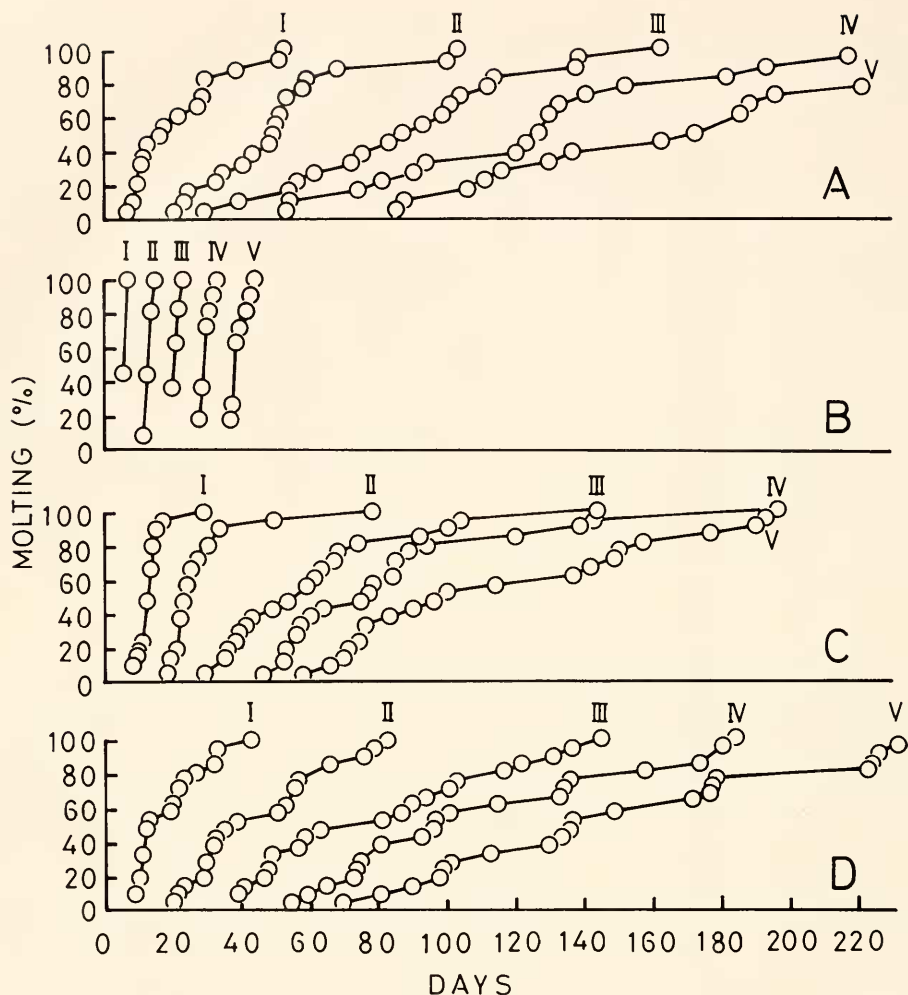


FIGURE 1. Molting percentage of surgically treated crayfish, specimens of *Procambarus clarkii*, at five consecutive molts after the initial laboratory molt. A, untreated crayfish (control); B, both eyestalks removed; C, three pairs of walking legs removed; D, both uropods removed. In the groups where legs or uropods were removed, the regenerated legs or uropods were removed again one day after the third molt. Roman numerals I, II, III, IV and V, show the 1st, 2nd, 3rd, 4th, and 5th molt, respectively.

Large variation, however, was observed among the individuals. For example, in the first molt the earliest crayfish molted on the seventh day but the last crayfish molted on the fifty-third day. As clearly shown in Figure 1, this variation became more pronounced in the later molts.

Eyestalkless crayfish. Twelve of 23 treated crayfish died between the third and fifth molts. The remaining 11 completed all five consecutive molts within 43 days. T_{50} from the first to the fifth molt was 6 (35.3% compared to the value of

control animals), 13 (26.0%), 20.5 (23.6%), 29 (22.7%), and 39 (23.8%) days, respectively. The first molt occurred on the fifth day and all the treated individuals molted by the eighth day. All 23 crayfish completed the second molt between 6 and 8 days after the first molt and finished the third molt between 7 and 8 days after the second molt. Two animals, however, died on the first and fifth day respectively, after the third molt.

The surviving crayfish completed the fourth molt by the intermolt cycle of 7 to 12 days. Seven animals died within two days after the fourth molt. The 14 survivors completed the fifth molt by the intermolt cycle of 8 to 12 days. Three died at the time of the fifth molt.

Legless crayfish. Twenty of 21 animals completed five consecutive molts (one animal completed only four). T_{50} from the first to the fifth molt was 13 (76.5% of the control value), 24 (48.0%), 55 (63.2%), 77 (60.2%), and 99 (60.4%) days, respectively.

A few animals had longer molt cycles than the others. Roughly speaking, the operated animals could complete five molts during the time in which the untreated crayfish completed four molts. The walking legs regenerated to normal size after the second molt. Two crayfish died; one at the time of its third molt and another at its fourth molt.

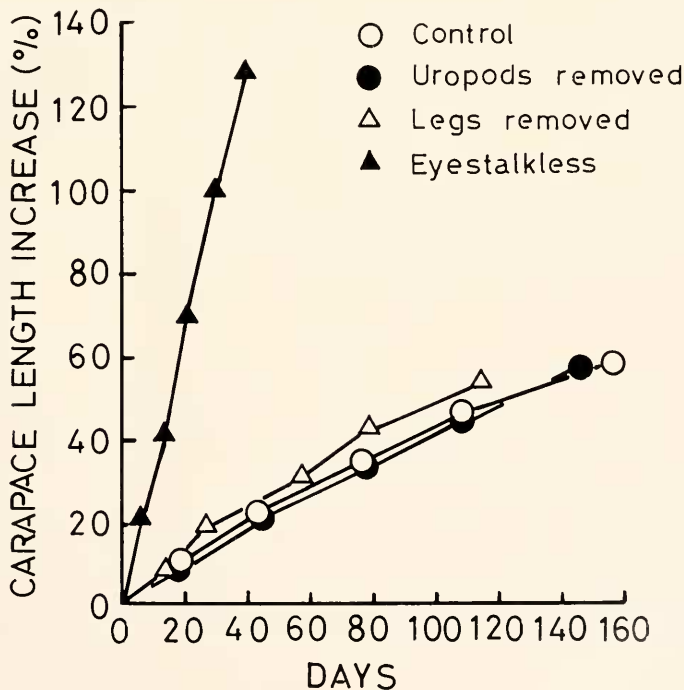


FIGURE 2. The average percentage of increase in carapace length of specimens of the crayfish, *Procambarus clarkii*, to the original length after each of the five consecutive molts. Each point represents the mean days required for each molt following the initial laboratory molt.

TABLE II

The average carapace length (mm) after each molt in crayfish, Procambarus clarkii.

	Initial	1st	2nd	3rd	4th	5th	Mean
Control	11.1 ± 0.2	12.3 ± 0.3 (10.5)	13.5 ± 0.4 (10.4)	14.9 ± 0.5 (10.2)	16.1 ± 0.5 (8.2)	17.5 ± 0.6 (8.5)	(9.6)
Eyestalkless	11.5 ± 0.3	13.9 ± 0.3 (20.7)	16.3 ± 0.4 (17.5)	19.5 ± 0.5 (19.8)	22.8 ± 0.6 (16.8)	26.2 ± 0.7 (14.7)	(17.9)
Legless	11.2 ± 0.3	12.1 ± 0.3 (8.1)	13.3 ± 0.3 (10.0)	14.6 ± 0.4 (10.2)	15.9 ± 0.4 (8.6)	17.1 ± 0.5 (7.8)	(8.9)
Uropodless	11.6 ± 0.3	12.7 ± 0.3 (8.9)	14.1 ± 0.5 (11.3)	15.5 ± 0.6 (9.7)	16.8 ± 0.7 (8.6)	18.3 ± 0.9 (8.6)	(9.4)

The numerals in parentheses are the average percentage of increased carapace length for each molt.

Uropodless crayfish. Twenty of 21 animals completed five consecutive molts, one animal staying at the fourth molt during the experimental period. T_{50} from the first to the fifth molt was 12 (70.0% of the control value), 39 (78.0%), 73 (83.9%) 96 (75.0%), and 136 (82.9%) days, respectively. Regeneration of the uropods was observed on all crayfish after the second molt. Two crayfish died; one at the first molt and another at the third molt.

Growth rates

The mean values of growth rate for crayfish which completed five molts were measured after each of five consecutive molts with special reference to the mean value of the initial carapace length, which was measured after the initial laboratory molt (Fig. 2). As is clear from Figure 2, the rate of increase of carapace length of the eyestalkless group is more than six times greater than that of the control group.

The percentage increases in carapace length from between each premolt and corresponding postmolt stage are shown in Table II. The average percentage of increase in carapace length per molt was 9.6% for untreated group, 17.9% for eyestalkless group, 8.9% for legless group, and 9.4% for uropodless group.

DISCUSSION

The results suggest that the removal of eyestalks, legs, or uropods stimulates molt in the crayfish, *Procambarus clarkii*. The results support the findings of other authors studying eyestalkless crayfish, *Cambarus clarkii* (Smith, 1940), fiddler crabs, *Uca pugnator* (Abramowitz and Abramowitz, 1940) and land crabs, *Gecarcinus lateralis* (Skinner and Graham, 1972); and walking legless crayfish, *Procambarus clarkii* (Bittner and Kopanda, 1973), freshwater shrimp, *Palaemonetes kadiakensis* (Stoffel and Hubschman, 1974) and land crabs, *Gecarcinus lateralis* (Skinner and Graham, 1970). Brown and Cunningham (1939) and Smith (1940) discussed the possible mechanisms concerning the effects of eyestalk removal on the molt, suggesting that the eyestalks contain the inhibiting hormone which delays molting.

Abramowitz and Abramowitz (1940) found that in fiddler crabs both molt and growth were stimulated by eyestalk removal. In our experiments, we found that the average growth rate of carapace length in the eyestalkless crayfish after every molt was about twice that of the intact animals, and that the duration of the molt

cycles of the former was about one fourth of that of the latter. From these data, the mean weight increase of eyestalkless animals was about 15 times larger than the corresponding weight of the control animals 40 days after the operation. On the other hand, the molts of both the leg- or uropod-removed crayfish were stimulated, but the carapace length growth rate after every molt was less than that of the controls. Thus, it is clear that growth was accelerated by eyestalk removal, and that the secretion of molting hormone was induced by the growth of the body.

Weis (1976) found that the removal of seven legs from a fiddler crab did not result in a significant increase in carapace width after molt and regeneration. Krishnakumaran and Schneiderman (1970) found that ecdysterone did not increase DNA synthesis in the epidermis, muscles, nerve cells, and connective tissue of crayfish, *Procambarus clarkii*, although it induced molting. In the present experiments, the carapace length increased after molt in crayfish whose legs or uropods were removed, but the rate of increase was less than that of the untreated control.

It may be safely concluded that the removal of legs or uropods has the same effects on molt and growth in crayfish. This would support the hypothesis of Skinner and Graham (1972) that molt inhibitory factors do not exist in the limbs of Crustacea and that precocious molt is stimulated by the severing of a critical number of nerves. Stoffel and Hubschman (1974) pointed out that the loss of several walking legs stimulates the neurosecretory cells of the X-organ via nervous impulses to stop releasing the molt-inhibiting hormone. According to Holland and Skinner (1976), the removal of one or more limb buds of *Gecarcinus lateralis* inhibited growth of the remaining limb buds until re-regenerates reached an appropriate size. In the present experiment, both legless or uropodless crayfish molted before they grew in body size sufficient for molt. Therefore, if the crayfish is missing legs or uropods, it may be possible that the regenerating buds grow faster than all the other parts of the body, and the regenerating buds stimulate the release of molt hormone. The intensity of stimulus may depend, to some extent, on the number of buds, or on the surface of the cut ends or organs. It is known that land crabs missing many legs prepare for molt sooner than those which are missing one or two legs (Skinner and Graham, 1970).

It has been reported that the mortality is high in destalked crustaceans (Abramowitz and Abramowitz, 1940; Smith, 1940; Skinner and Graham, 1972). According to Abramowitz and Abramowitz (1940), the viability is concerned in some way with the eyestalks. In general, death during molt by intact Crustacea is common. In the present experiments, five crayfish of the control group, two of the legless group, and two of the uropodless group died at the time of molt or within a day after their molt. In the eyestalkless group, twelve individuals died at the time of molt or within a few days after their molt. The mortality of the eyestalkless group, therefore, is higher than the other groups. It may be possible that the eyestalkless crayfish grow too rapidly to prepare properly for molt and this leads to failure at molt.

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SUMMARY

1. Removal of a pair of eyestalks induces precocious molt and accelerates the growth of the crayfish, *Procambarus clarkii*.
2. Removal of three pairs of walking legs or a pair of uropods induces precocious molt without any effects on the growth of the body.
3. The average time required for 50% of the individuals to reach the fifth molt after the initial molt is 39 days for eyestalkless crayfish, 99 days for legless, and 136 days for uropodless crayfish. The untreated crayfish (controls) required 164 days to attain the fifth molt.
4. The average percentage of increase in carapace length at the time of the fifth molt is 9.6% for the untreated crayfish, 17.6% for eyestalkless, 8.9% for legless, and 9.4% for the uropodless group.
5. The mortality during the approximately eight-month experimental period was 5:23 for the untreated group, 12:23 for the eyestalkless group, 2:23 for the legless group and 2:23 for the uropodless group. The eyestalkless crayfish were healthy until the third molt, but experienced great mortality at the time of the fourth and fifth molt. The failure of molt in eyestalkless crayfish may be due to too rapid increase in the body size, impairing preparations for molt.

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